Propagation of *Anemone* x *hybrida* by Root Cuttings[1]

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Abstract

Stock plants of *Anemone* x *hybrida* Paxton 'Honorine Jobert' and 'Richard Ahrens' were grown in 3.8 liter (#1) containers for 30 weeks beginning in April, and fertilized daily with a complete nutrient solution providing 10, 40, 80 or 150 mg/liter (ppm) nitrogen (N), in a constant ratio of 1 ammonium: 2 nitrate. At the end of 30 weeks (November), 4-cm-long root cuttings were harvested from the stock plants and treated with the potassium salt of indolebutyric acid (K-IBA) at 0, 100, 500 or 1000 mg/liter (ppm), then placed in cell packs containing a pine bark-based substrate, one cutting per cell. The containers were arranged under intermittent mist in a heated greenhouse. Overall, 91% of the cuttings regenerated a complete plant. There were cultivar differences in regeneration, and the highest K-IBA concentration was inhibitory to 'Honorine Jobert'. Nitrogen nutrition of the stock plants, K-IBA treatment of the root cuttings, and fresh weight of the root cuttings, had no significant effect on percent regeneration. Time to shoot emergence was reduced by higher rates of N applied to the stock plants, and increased at the highest concentration of K-IBA in 'Honorine Jobert', but not in 'Richard Ahrens'. Dry weights of the regenerated plants increased with increasing weight of the cuttings from which they originated. They were related linearly to rate of N applied to the stock plants in 'Honorine Jobert', and quadratically in 'Richard Ahrens', with maximum plantlet weight predicted at 114 mg/liter (ppm) N. At the observed optimal rate of N applied to the stock plants, dry weights of the regenerated plants increased with increasing K-IBA concentration, in a quadratic manner. Maximum plantlet weight is predicted at 459 mg/liter (ppm) K-IBA in 'Honorine Jobert', and at 425 mg/liter (ppm) in 'Richard Ahrens'.

Index words: anemone, Ranunculaceae, ornamentals, perennials, mineral nutrition, K-IBA.

Significance to the Nursery Industry

Fall flowering anemones (Ranunculaceae) are highly sought after perennials whose widespread acceptance in the landscape has been hampered by propagation difficulties. In a survey of perennial propagation firms across the United States conducted by the authors in October 1997, fall flowering anemones were the plant most often cited as needing improvement in propagation. Currently, propagation is accomplished by division or root cuttings, with very low multiplication rates in either case. Published information regarding this topic is limited to general recommendations on handling of root cuttings, based on scant research involving woody species. However, under the conditions described herein, between 90% and 100% of root cuttings of *Anemone* x *hybrida* Paxton can produce a complete, viable plantlet. Weight of the root cuttings, nitrogen (N) nutrition of the stock plant, and treatment of the root cuttings with the potassium salt of indolebutyric acid (K-IBA) can all be optimized to increase plantlet size.

Introduction

Fall flowering anemones, often referred to as 'Japanese anemones', comprise four closely related species: *A. hupehensis* Lemoine, including *A. hupehensis* var. *japonica* (Thunb.) Bowles & Stearn, *A.* x *hybrida*, *A. tomentosa* (Maxim.) S.J. Pei, and *A. vitifolia* Buch.-Ham. The name *A.* x *hybrida* is applied properly only to plants originating from the cross of *A. hupehensis* x *A. vitifolia*. A survey by the authors of 32 propagators of perennials indicated that among the four species, *A.* x *hybrida* consistently presents the greatest propagation difficulties.

In the landscape, fall flowering anemones are of exceptional value. Grown in partial shade, they are robust, adaptable plants within the limits of USDA cold hardiness zones 5 to 8. Their foliage remains attractive from its emergence in spring, to the first frost, and their elegant flowers are produced in profusion at a time of year when few other perennials are in flower. Once established, they spread, sometimes aggressively, by means of shoots developing in great numbers along the entire lengths of the roots (1, 7, 12). Producers in the United States are unable to meet demand, due to propagation difficulties. Most plants of *A.* x *hybrida* sold in the United States are grown from bareroot field divisions produced in The Netherlands.

Propagation of *A.* x *hybrida* by root cuttings is generally recommended in the literature, with suggestions regarding the best time of year for propagation varying widely (1, 5, 6, 9, 12, 13). Crown division yields few new plants per stock plant (12), and at present no micropropagation (tissue culture) protocols have been reported. Propagators surveyed by the authors reported poor success with root cuttings harvested at any time of the year. They indicated that those root cuttings that produce shoots often do not regenerate feeder roots, resulting in death of the cutting. This suggests that auxin treatment might lead to better plantlet survival by stimulating feeder root development. Ideally, propagators would prefer that plantlets be produced in bedding plant type trays from container-grown stock plants, with 90% of the cuttings resulting in plantlets.

Research on propagation of various species by root cuttings has been limited, and involves mostly woody plants (3, 14). Several studies have shown a strong positive correlation between high N levels in the growing substrate of stock plants, and both the number of shoot buds produced on roots of *Chondrilla juncea* L. (skeleton weed) and *Euphorbia esula* L. (leafy spurge), and their ability to regenerate whole plants

[1]Received for publication September 27, 1999; in revised form February 7, 2000. This research was funded in part by the North Carolina Agricultural Research Service (NCARS), Raleigh, NC 27695-7643, and by a grant from the Perennial Plant Association, 3383 Schirtzinger Road, Hilliard, OH 43026. Special thanks to Joy M. Smith and William H. Swallow for statistical guidance, and Juan R. Acedo and William M. Reece for technical assistance. From a thesis submitted by J.-J.B.D. in partial fulfillment of the requirements for the MS degree.

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(8, 10, 11). In stem cuttings of various plants, however, adventitious root formation has been shown to be affected negatively by high N nutrition of stock plants (2).

Therefore, the following research was undertaken with two objectives. The first objective was to determine the effect of N nutrition of stock plants and subsequent K-IBA treatment of root cuttings on development and establishment of complete plants of two cultivars of fall flowering anemone. The second objective was to provide the basis for a practicable system for production of plantlets of anemone in small cell containers with a high success rate. The water soluble auxin K-IBA was used in this research to preclude the potential for injury to root tissue from an organic solvent needed to dissolve the free acid of IBA.

Materials and Methods

Culture of stock plants. On March 9, 1998, 64 #1 field divisions each of cultivars 'Honorine Jobert' and 'Richard Arhens' anemone were potted in 3.8-liter (#1) containers filled with a substrate of composted pine bark:sand (8:1 by vol), amended with 2.4 kg/m^3 (4 lb/yd^3) dolomitic limestone and 0.9 kg/m^3 (1.5 lb/yd^3) Micromax micronutrient fertilizer (The Scotts Co., Marysville, OH.). Plants were placed in an unheated cold frame and irrigated with tap water as needed until April 17, when they were moved to a gravel container pad.

Starting on April 17, 400 ml (0.6 in) of a complete nutrient solution was applied daily to each container via pressure compensated spray stakes (Acu-Spray Stick, Wade Mfg. Co., Fresno, CA) at a rate of 200 ml/min (0.3 in/min). Irrigation volume was increased as needed to maintain a 0.25 leaching fraction throughout the study. The solution provided four N concentrations [10, 40, 80 or 150 mg/liter (ppm)], with a constant ratio of 1 ammonium: 2 nitrate. Concentrations of P, K, Ca, and Mg were 50, 150, 191, and 48 mg/liter (ppm), respectively, for all N treatments. When necessary, pH of the solutions was adjusted to 6.0 using HCl. Fifty percent shade was imposed after 16 weeks, using nylon mesh shade cloth, until harvest of the cuttings.

Propagation. Thirty weeks after initiation of the study, roots were washed free of substrate and processed into root cuttings 4 cm in length. The cuttings were randomized as to the presence or absence of visible shoot buds. Cuttings representing each stock plant fertilization rate were weighed individually, then immersed for 5 sec in K-IBA at 0, 100, 500 or 1000 mg/liter (ppm). The treated cuttings were placed horizontally, one per cell, in packs of six cells [cell vol = 160 cm^3 (9.8 in^3)] containing pine bark amended with 2.4 kg/m^3 (4 lb/yd^3) dolomitic limestone. They were covered with 1.5 cm (0.6 in) substrate. The packs were in turn placed in 25 × 51 cm (10 × 20 in) (#1020) flats, six packs per flat, and after 24 hr, drenched with a fungicide [Physan 20 (n-alkyl dimethyl benzyl ammonium chloride 10% + n-alkyl ethylbenzyl ammonium chloride 10%)] at 2.6 ml/liter (0.3 oz/gal). Flats (64 total) were placed under intermittent mist in a heated greenhouse under natural photoperiod and irradiance with days/nights of 24 ± 1.7C (75 ± 3F)/20 ± 1C (68 ± 2F). Mist was applied via a gantry mounted travelling spray boom (ITS, McConkey Co., Sumner, WA) with continuous adjustment of frequency as a function of relative humidity, and travelling speed set at 15 m/min (50 ft/min). This setting resulted in the substrate surface just reaching dryness before being misted again.

The experiment was a randomized complete block design with a factorial arrangement of treatments: two cultivars, four rates of N applied to the stock plant, and four rates of K-IBA. There were six replications with six cuttings per replication.

One week and 2 weeks after cuttings were inserted into cells, and subsequently every Monday and Thursday, shoot emergence data were recorded. Cumulative percent emergence was calculated for each experimental unit on each of those days. After 8 weeks, misting was discontinued, and plantlets were irrigated overhead every 3 days. After 12 weeks, roots of the resulting plantlets were washed free of substrate, and separated from the shoots. Roots and shoots were dried for 96 hr at 70C (160F) and weighed. Data were subjected to analysis of variance and regression analysis where appropriate. The effects of cutting weight were analyzed by analysis of covariance, with cutting weight as the covariate.

Results and Discussion

Percent regeneration. Percent regeneration across cultivars and treatments, as measured by the proportion of root cuttings that resulted in a plantlet, was 91%. There was a significant difference between the two cultivars ($P < 0.0001$), with 'Honorine Jobert' averaging 84% across all treatments, and 'Richard Ahrens', 98%.

Weight of the root cutting and rate of N applied to the stock plant did not affect percent regeneration in either cultivar. Response to K-IBA was quadratic in 'Honorine Jobert', with a maximum of 90% regeneration predicted at 240 mg/liter (ppm) K-IBA, and 1000 mg/liter (ppm) K-IBA having a detrimental effect on percent regeneration (Fig. 1). Percent regeneration was unaffected by K-IBA in 'Richard Ahrens' (data not presented).

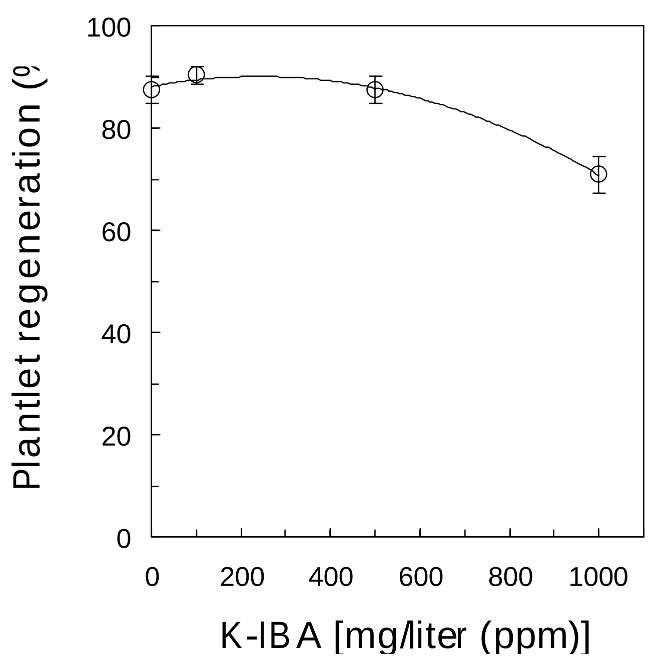

Fig 1. Effect of K-IBA treatment on percent regeneration of 'Honorine Jobert' anemone. Each point represents the mean of 24 observations. Vertical bars are the standard error of the mean. R^2 = 0.99.

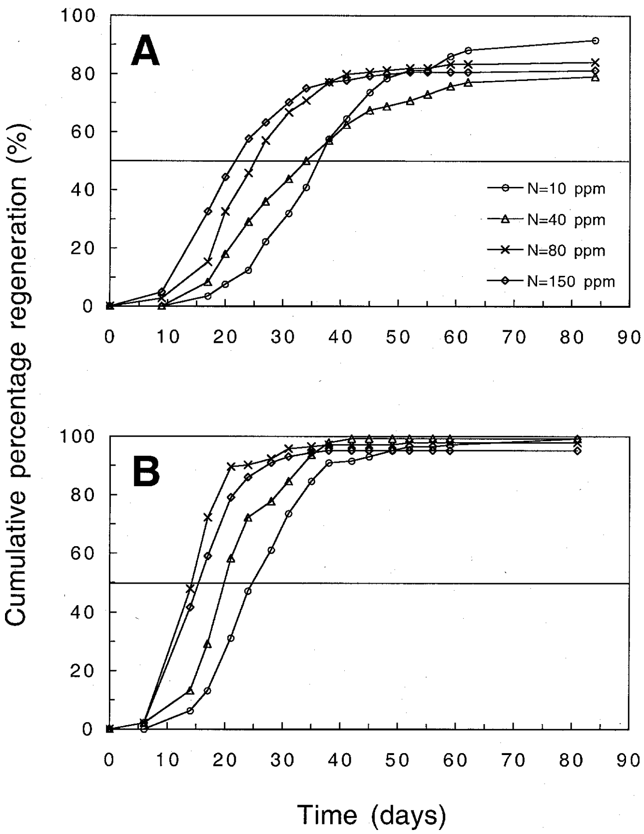

Fig 2. Effect of rate of N applied to stock plants of *Anemone* x *hybrida* on cumulative percent shoot emergence over time. Each point represents the mean of 24 observations. (A) 'Honorine Jobert', and (B) 'Richard Ahrens'.

Time to emergence. Cultivars differed markedly in time to 50% emergence, with 'Honorine Jobert' reaching 50% emergence in 30 days, and 'Richard Ahrens' in 20 days (data not presented). Cutting weight did not affect time to 50% emergence (data not presented). Increasing rate of N applied to the stock plant shortened time to emergence in both cultivars (Fig. 2). The shortest mean time to 50% emergence in 'Honorine Jobert' was 22 ± 1 days, at 150 mg/liter (ppm) N. It was 16 ± 0.5 days in 'Richard Ahrens', at 80 mg/liter (ppm). The relationship between time to 50% emergence and rate of N was linear in 'Honorine Jobert' and quadratic in 'Richard Ahrens' (Fig. 3). For this cultivar, the fastest emergence (15 days) is predicted to occur at 114 mg/liter (ppm) N. 'Richard Ahrens' was unaffected by K-IBA (data not presented), whereas increasing K-IBA concentration delayed emergence in 'Honorine Jobert' (Fig. 4). This cultivar showed a quadratic increase in time to 50% emergence with increasing K-IBA concentration, with an observed maximum mean time to 50% emergence of 38 ± 2 days, at 1000 mg/liter (ppm) K-IBA.

Plantlet dry weight. Shoot dry weight, root dry weight, and plantlet dry weight responded similarly. Therefore, only plantlet dry weight is presented. Overall mean plantlet weight was 246 mg (0.009 oz) for 'Honorine Jobert', and 362 mg (0.013 oz) for 'Richard Ahrens'.

Cutting fresh weight had a strong positive influence on dry weight of the plantlet produced (Fig. 5). There was a small difference between cultivars in the slope of the covariance adjustment for weight of the cutting, and no difference between rates of N, within each cultivar. The value of the slope was 0.92 ± 0.10 for 'Honorine Jobert' and 1.14 ± 0.09 for 'Richard Ahrens'. From this, we concluded that within the range used in this experiment, each unit increase in cutting fresh weight will result in approximately one unit increase in the dry weight of the plantlet produced from that cutting.

Plantlet dry weight increased linearily with increasing N in 'Honorine Jobert', and responded quadratically in 'Richard Ahrens', with maximum plantlet weight predicted at 115 mg/liter (ppm) N (Fig. 6). The largest mean dry weight by rate of N was 358 ± 17 mg for 'Honorine Jobert', observed at 150 mg/liter (ppm) N, and 469 ± 16 mg for 'Richard Ahrens', at 80 mg/liter (ppm) N.

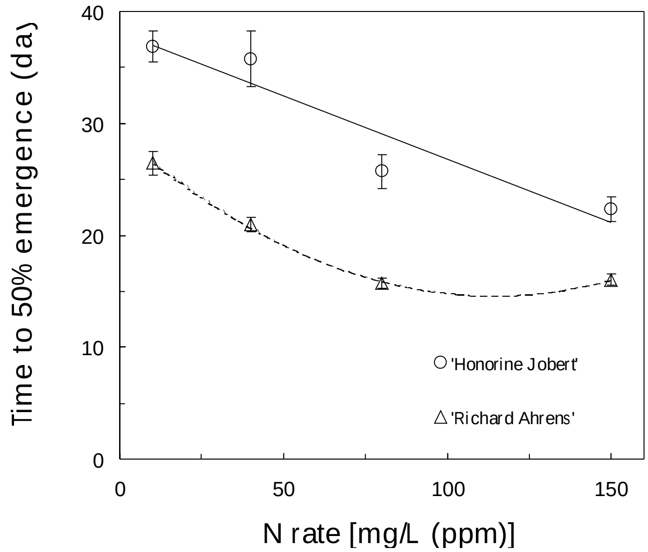

Fig. 3. Effect of rate of N applied to stock plants of *Anemone* x *hybrida* on time to 50% shoot emergence. Each point is the mean of 24 observations. Vertical bars are the standard error of the mean. 'Honorine Jobert' R^2 = 0.89. 'Richard Ahrens' R^2 = 0.99.

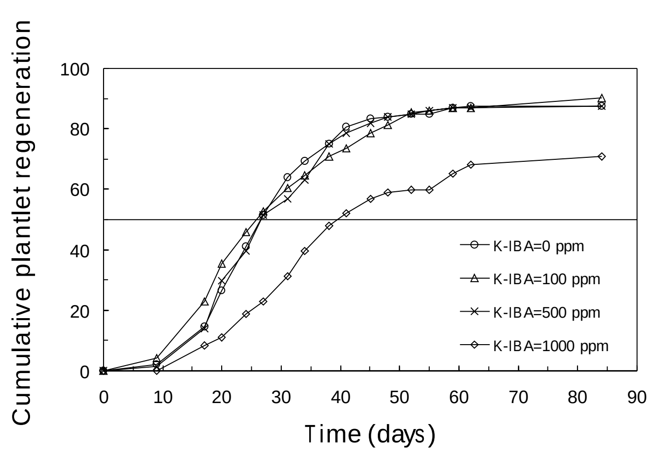

Fig. 4. Effect of K-IBA on cumulative percent shoot emergence over time in 'Honorine Jobert' anemone. Each point represents the mean of 24 observations.

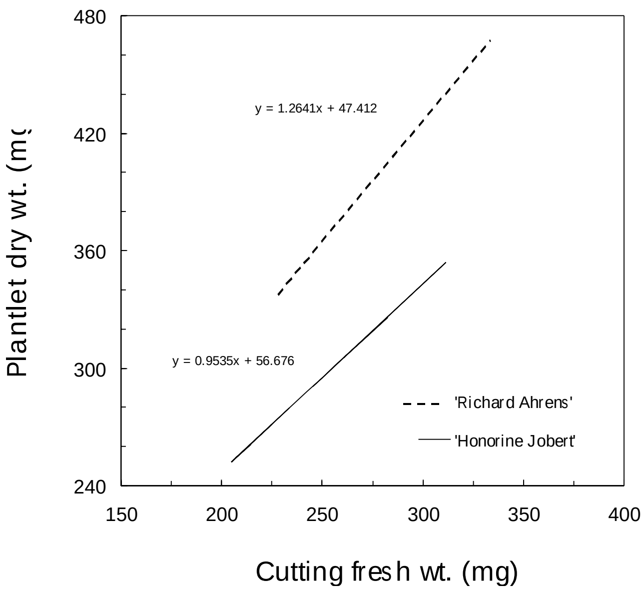

Fig. 5. Effect of weight of root cuttings of 'Honorine Jobert' and 'Richard Ahrens' anemone on weight of plantlets regenerated. Lines are the covariance adjustment function for each cultivar.

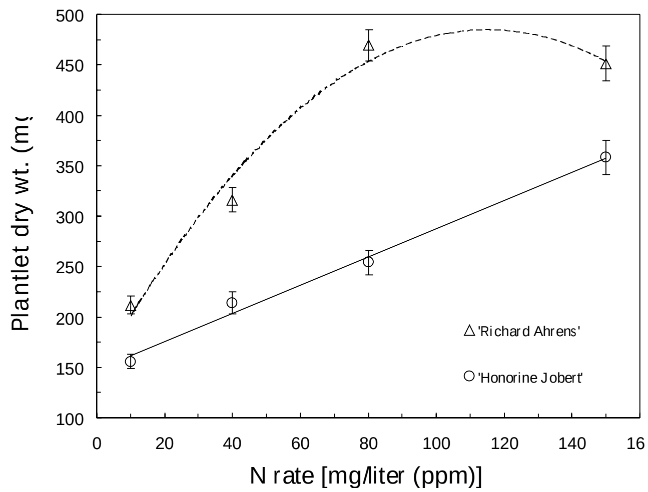

Fig. 6. Effect of rate of nitrogen applied to stock plants of *Anemone* x *hybrida* on dry weight of regenerated plantlets. Each point represents the mean of 114 to 133 observations for 'Honorine Jobert', and 136 to 143 observations for 'Richard Ahrens'. Vertical bars are the standard error of the mean. 'Honorine Jobert' R^2 = 0.99. 'Richard Ahrens' R^2 = 0.98.

At those respective rates of N, plantlet dry weight responded to K-IBA in a quadratic manner in both cultivars (Fig. 7). However, there was no overall interaction between rate of N and K-IBA. Maximum response is predicted to occur at 459 mg/liter (ppm) K-IBA in 'Honorine Jobert', and at 425 mg/liter (ppm) in 'Richard Ahrens'. At these concentrations, a 37% increase in plantlet dry weight would be expected in 'Honorine Jobert', compared to using no K-IBA. In 'Richard Ahrens', the increase would be expected to reach 13%. At those concentrations, K-IBA would not affect percent regeneration or rate of shoot emergence.

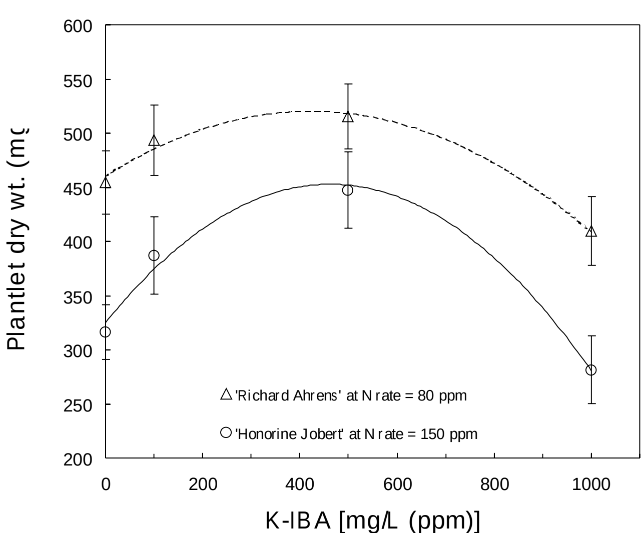

Fig. 7. Effect of K-IBA on plantlet dry weight of *Anemone* x *hybrida*. Each point represents the mean of five observations, with each observation consisting of six root cuttings. Vertical bars are the standard error of the mean. 'Honorine Jobert', at rate of N applied to the stock plant = 150 mg/liter (ppm). R^2 = 0.98. 'Richard Ahrens', at rate of N applied to the stock plant = 80 mg/liter (ppm). R^2 = 0.98.

In conclusion, when propagating *A.* x *hybrida*, the weight of the root cutting does not influence whether or not a plantlet can be regenerated, and how rapidly, but larger cuttings will yield larger plantlets. Visible shoot buds are not an indicator of regenerative potential, since cuttings regenerated plantlets regardless of the presence or absence of visible buds. Optimal nitrogen nutrition of the stock plant will shorten the time to regeneration, and the plantlet produced will attain a larger size in the same length of time. However, the optimal rate of N varies by cultivar, and has no relation with the percentage of cuttings that produce a plantlet. To maximize multiplication rates, total amount of cutting material generated by each stock plant should also be taken into consideration.

In a study of N nutrition of *A.* x *hybrida*, Dubois (4) reported the greatest production of root cutting material for the cultivar 'Margarete' occurred in stock plants that were supplied with 119 mg/liter (ppm) N. This rate was somewhat lower than the optimal rate for top growth [143 mg/liter (ppm)]. Finally, treatment with K-IBA, at low concentrations appears helpful in increasing the size of the resulting plantlet in some cultivars. As K-IBA concentration increased, however, both percent regeneration and time to production of a shoot may be adversely affected, again dependent on cultivar.

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